Autophagy Impairment in Parkinson's Disease

Cansu Karabiyik[1], Min Jae Lee[2]* and David Rubinsztein[1,3]*

[1]Cambridge Institute for Medical Research, Department of Medical Genetics, Cambridge Biomedical Campus, Wellcome Trust/MRC Building, Cambridge Biomedical Campus, Hills Road, Cambridge, CB2 0XY, UK.

[2]Department of Biochemistry and Molecular Biology, Seoul National University College of Medicine, Seoul 03080, Korea.

[3]UK Dementia Research Institute, Cambridge Biomedical Campus, Cambridge Biomedical Campus, Hills Road, Cambridge, UK.

*E-mail address for correspondence: dcr1000@cam.ac.uk (DCR), minjlee@snu.ac.kr (MJL)

Abstract

Parkinson's disease (PD) is a debilitating movement disorder typically associated with the accumulation of intracytoplasmic aggregate-prone protein deposits. Over recent years, increasing evidence has led to the suggestion that the mutations underlying certain forms of PD impair autophagy. Autophagy is a degradative pathway that delivers cytoplasmic content to lysosomes for degradation and represents a major route for degradation of aggregated cellular proteins and dysfunctional organelles. Autophagy upregulation is a promising therapeutic strategy that is being explored for its potential to protect cells against the toxicity of aggregate-prone proteins in neurodegenerative diseases. Here we describe how the mutations in different subtypes of PD can affect different stages of autophagy.

Abbreviations

AD, Alzheimer's disease; AMPK, AMP activated protein kinase; ATG, autophagy related genes; CMA, chaperone-mediated autophagy; FIP200, focal adhesion kinase family interacting protein of 200 kDa; GBA,

glucocerebrosidase; HD, Huntington's disease; MTOC, microtubule organizing center; mTORC1, mammalian target of rapamycin complex 1; PD, Parkinson's disease; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol 3-phosphate; SNCA, synuclein; TFEB, transcription factor EB; ULK1, mammalian homologs of the *C. elegans* uncoordinated-51 kinase 1; WIPI, WD repeat domain phosphoinositide-interacting protein.

Introduction

Autophagy is an evolutionarily conserved degradation pathway responsible for digestion and recycling of most long-lived intracytoplasmic proteins and organelles. Autophagy is subcategorized into three types – microautophagy, chaperone-mediated autophagy (CMA) and macroautophagy. Microautophagy involves degradation of cytosolic contents via small invaginations in the lysosome membrane (1), while CMA involves selective translocation of cytoplasmic proteins with a KFERQ-like peptide motif into the lysosomal lumen for degradation (2). Macroautophagy (hereafter called autophagy and the focus of this review) is characterized by engulfment of cytoplasmic cargo by a double-membraned cup-shaped transient structure known as the phagophore. After the phagophore expands and its edges close, the resulting vesicle is known as an autophagosome. Autophagosomes then fuse with lysosomes resulting in degradation of the autophagosomal contents (Figure 1). This series of events is regulated by an array of proteins called autophagy-related (ATG) proteins.

It has become evident in recent years that both selective and non-selective types of autophagy exist and maintain degradation of cargo material, such as aggregate-prone proteins (aggrephagy) and damaged organelles, such as mitochondria (mitophagy), peroxisomes (pexophagy) and endoplasmic reticulum (ER-phagy) (3). Mutations underlying various neurodegenerative diseases that manifest with intracytoplasmic protein aggregates have been shown to affect the clearance of these substrates by compromising autophagy (4). In the present review, we focus on autophagy impairment in Parkinson's disease (PD). Autophagy upregulation has been studied extensively as a promising strategy for treatment of PD. Therefore, a more refined distinction between the classes of autophagy defects underlying certain subtypes of PD may be helpful in strategizing effective treatments for this condition.

The Autophagic Machinery

Autophagy is essential for cellular health and is activated as a response to the nutrient state of the cell. In basal conditions, the presence of nutrients, growth factors and AMP/ATP levels are sensed

by the Rag/Ragulator complex, tyrosine kinase receptors and AMP-dependent protein kinase (AMPK), leading to activation of the primordial negative autophagy regulator, mammalian target of rapamycin complex 1 (mTORC1). Active mTORC1 inhibits the ULK1-ATG13-FIP200 complex through phosphorylation of ULK1/2 and ATG13. The ULK complex is the most upstream unit amongst the autophagy proteins and plays a crucial role in autophagy initiation. The inhibition of this complex results in suppression of autophagosome formation (5–7).

During cellular starvation, autophagy activation provides building blocks from degraded macromolecules. As a response to low nutrient levels, mTORC1 is inhibited leading to dephosphorylation of the ULK complex, causing activation of the class III PI3-kinase, VPS34. VPS34 is recruited to the phagophore initiation site where it interacts with VPS15, ATG14, and Beclin 1 to generate phosphatidylinositol 3-phosphate (PI3P), an essential lipid component of the autophagosomes. The presence of PI3P on nascent autophagosomes facilitates the recruitment of PI3P-binding proteins, such as WD repeat domain phosphoinositide-interacting protein 2 (WIPI2), which is crucial for recruitment of downstream autophagic proteins (8–10).

Next, phagophore elongation occurs, mediated by two specialized ubiquitin-like conjugation systems, with membranes obtained from the endoplasmic reticulum, Golgi, mitochondria and plasma membrane (11–13). The first system involves a conjugation reaction between ATG12 and ATG5 mediated by the E1- and E2-like enzymes ATG7 and ATG10, respectively. The subsequent non-covalent binding between ATG12-ATG5 complex and ATG16L1 enables the resulting complex to associate with pre-autophagosomal membranes to assist their elongation by recruiting LC3 and its family members. Membrane association of LC3 is mediated by the second conjugation system. After the C termini of LC3 family members are cleaved by ATG4, exposing glycine residues, LC3 family proteins can be conjugated to phosphatidylethanolamine (PE) in the preautophagosomal membranes via a reaction mediated by ATG7, ATG3, and ATG12-ATG5-ATG16L1. The lipid-conjugated LC3 is known as LC3-II. The transmembrane protein mATG9 may assist in the expansion of the phagophore by providing further lipids (14,15).

Following the completion of autophagosome formation, these vesicles are transported along microtubules to the microtubule organizing center (MTOC), where lysosomes are clustered. This facilitates autophagosome-lysosome fusion, which results in the degradation of the autophagosomal contents by lysosomal acid hydrolases. The expression of lysosomal enzymes is

regulated by the transcription factor EB (TFEB), which is the master regulator of autophagy and lysosomal genes. Translocation of TFEB to the nucleus enables its transcriptional activity, autophagosome formation and autophagosome-lysosome fusion (16). Studies have shown that overexpression of TFEB improves degradation of complex molecules (17,18).

Autophagy and Parkinson's Disease

Basal autophagy is essential for maintaining neuronal homeostasis, since neurons are particularly susceptible to the accumulation of defective organelles and proteins due to their postmitotic nature. The importance of autophagy for the nervous system was confirmed by studies in which suppression of autophagy in *atg7*- and *atg5*-deficient mice led to phenotypes similar to those observed in neurodegenerative diseases, such as progressive motor impairment and intracytoplasmic inclusion bodies (19,20). This supports earlier studies showing the importance of autophagy in the clearance of aggregate-prone proteins in models of PD (21). Consistent with this, *in vivo* and *in vitro* studies, described below, have shown that autophagic dysfunction plays a likely role in disease pathogenesis.

PD is the most common neurodegenerative movement disorder. It is characterized by motor deficits and non-motor symptoms, like mood disorders and cognitive impairment. PD pathology is characterised by progressive neuronal loss which is observed in many areas but is most marked in dopaminergic neurons in the substantia nigra, and the presence of intraneuronal inclusions known as Lewy bodies (LBs) and Lewy neurites enriched with filamentous forms of α-synuclein (α-syn) (22,23). This protein, encoded by the *SNCA* gene, has been extensively studied. Multiplications of the *SNCA* locus cause autosomal dominant forms of PD. The identification of familial cases with *SNCA* multiplications revealed a strong correlation between levels of α-syn and disease severity (24). Although these findings show that increased levels of α-syn is critical for disease severity, α-syn mRNA levels do not consistently change in sporadic PD cases. Since α-syn degradation is maintained by multiple degradative routes including autophagy (21), chaperone-mediated autophagy (CMA) (25) and the endolysosomal pathway (26), impaired clearance of α-syn is suggested to be an underlying mechanism responsible for α-syn accumulation and aggregation in sporadic cases of PD.

Initial genetic studies led to the discovery of causal mutations in PD, which were subjected to extensive research and many were found to have functions related to autophagy. These can be subdivided into mutations that affect mitophagy or cause trafficking or lysosomal defects (Figure 2). Consistent with the converging evidence pointing towards impaired autophagy in PD, a recent meta-analysis of genome-wide association studies (GWAS) has identified novel PD risk loci that may play roles in autophagy and lysosomal function (27).

Mitophagy defects in PD

Mitochondria are essential for maintaining critical cellular functions, such as generating energy. Genetic linkage studies identified mutations in phosphatase and tensin homologue-induced putative kinase 1 (PINK1 or PARK6) and Parkin (PARK2) in autosomal recessive juvenile Parkinson's disease. PINK1 and Parkin coordinately regulate the autophagic degradation of mitochondria (mitophagy), which is a crucial quality control that removes defective mitochondria from the cells, and these two proteins were first shown in *D. melanogaster* studies to act via the same pathway (28–30). Under normal conditions, the mitochondrial serine/threonine kinase PINK1 is recruited to the inner mitochondrial membrane (IMM) by the translocase of the outer membrane (TOM) complex and the translocase of the inner membrane (TIM23) complex. It is then cleaved by the mitochondrial processing peptidase (MPP) and presenilin-associated rhomboid-like protein (PARL) before it is translocated back to the cytoplasm. The cleaved PINK1 is ubiquitinated and consequently degraded by the 26S proteasome. However, when mitochondria are depolarised or damaged, PINK1 accumulates on the outer mitochondrial membrane (OMM), where it phosphorylates parkin and ubiquitin on Ser65, which in turn leads to activation of Parkin (31). Activated Parkin forms polyubiquitin chains on damaged mitochondria, which are then phosphorylated by PINK1. The phospho-polyubiquitination of dysfunctional mitochondria enables the binding of autophagy receptors, such as NDP52 and Optineurin, which serve as signals for autophagic clearance by recruiting LC3-containing phagophores (3,32,33).

The function of these PD-related mitophagy effectors have been subject to extensive research. Interestingly, deletion of *parkin* in mouse models did not cause an obvious behavioural phenotype (34–36), although it impaired striatal neuron mitochondria and caused a deficit in evoked dopamine release (37). *PINK1* deletion also resulted in striatal mitochondria defects and increased sensitivity to oxidative stress (38). However, a study in which *parkin* is deleted in adult mice described age-dependent loss of dopamine neurons, suggesting the existence of a compensatory mechanism in

germline knockouts (39). The importance of Parkin for ensuring mitochondrial quality was further shown in a study using the so-called mutator mice, in which mitochondrial function is gradually impaired due to an accelerated generation of mtDNA mutations. The absence of Parkin in the mutator mice led to degeneration of dopaminergic neurons. The motor deficit that appeared in the Parkin-mutator mice was reversed by administration of L-DOPA (40). A recent study in DA neurons derived from iPSCs with Parkin or PINK1 mutations show impaired ubiquitination of mitochondria and may explain how these mutations cause reduced clearance of damaged mitochondria (41).

Mutations in the F-box protein 7 (FBXO7) gene (PARK15) are associated with a severe form of juvenile onset PD. FBXO7 interacts with PINK1 and Parkin and helps facilitate Parkin-mediated mitophagy (42). PD-associated FBXO7 mutations in *D. melanogaster* led to mitochondrial toxicity as a result of FBXO7 aggregation in the mitochondria (43).

Trafficking defects in PD

It is well-established that α-syn plays a substantial role in PD pathogenicity. The presence of α-syn inclusion bodies affects autophagosome maturation and fusion with lysosomes, resulting in decreased protein degradation (44). *In vitro* studies show that the presence of α-syn inclusions specifically inhibits transport of endocytic and autophagic vesicles (45). Additionally, overexpression of α-syn *in vitro* and *in vivo* leads to compromised autophagosome biogenesis by inhibiting Rab1 causing mislocalization of mATG9 (46).

Autophagy is also impaired by the vacuolar protein sorting-associated protein 35 (VPS35) D620N mutation causing an autosomal dominant form of PD. VPS35 is a component of the retromer complex, which is required for the recruitment of the actin nucleation-promoting WASP and Scar homolog (WASH) complex to endosomes to facilitate protein sorting (47) and trafficking of transmembrane receptors within the endosome-to-Golgi retrieval pathway (48). D620N VPS35 associates poorly with the WASH complex and impairs the recruitment, which causes mATG9 mislocalization and inhibition of autophagosome formation (49). Interestingly, the effects of the VPS35 D620N mutation on autophagy were not mimicked by loss of VPS35 in this study and were unrelated to retrograde endosome-to-Golgi trafficking, suggesting that the point mutation may affect only a subset of VPS35 functions. Studies suggest that VPS35 interacts with Parkin (50), which in turn ubiquitinates VPS35 in human neuroblastoma cells (51). Interestingly, loss of Parkin led to decreased membrane association of VPS35 (52). Additionally, the D620N VPS35 mutation

has been suggested to affect the endolysosomal pathway (53) leading to increased α-syn aggregation (54). A study conducted in α-syn expressing mice showed a reduction in α-syn inclusions and reduced neuronal loss when delivering lentiviral WT VPS35, in contrast to lentiviral D620N VPS35 (55).

Lysosomal defects in PD

Degradation of α-syn can be maintained via multiple degradative routes. However, regardless of the route, functioning lysosomes are required for the degradation. The importance of lysosomal function for α-syn clearance was suggested by a study conducted in both cathepsin D (CatD) deficient mice and *C. elegans* showing that not only does CatD deficiency impair autophagy, but it also causes accumulation of α-syn aggregates in the nervous system (56). As outlined below, there is increasing evidence of lysosomal defects in PD.

Autosomal dominant mutations in the gene *LRRK2,* which encodes a multidomain protein with multiple functions, are among the most common genetic determinants underlying familial forms of PD. The causative role of LRRK2 in PD has been studied extensively and many studies suggest that LRRK2 is involved in endosome-to-lysosome trafficking. A study in *D. melanogaster* showed that the LRRK2 homolog, Lrrk, is localized on late endosomal and lysosomal membranes and that Lrrk physically binds to the late endosomal protein Rab7. Overexpression of mutant *GS lrrk*, analogous to the most common PD causing mutation in human *LRRK2* (G2019S), led to defective lysosomal positioning mediated by Rab7 (57). Further studies in *D. melanogaster* showed that loss of Lrrk function led to the accumulation of enlarged lysosomes containing undigested content, indicating defective lysosomal degradation. Constitutive activation of Rab9, which promotes endosomal recycling, suppressed the lysosomal dysfunction caused by the *G2019S lrrk* mutation (58). Additionally, endolysosomal sorting defects were observed in primary rodent neurons expressing G2019S LRRK2, leading to VPS35 retromer complex deficiency (59). The notion that LRRK2 is involved in regulating lysosome function is further supported by a study conducted in mice and in primary cells. In this study, expression of G2019S LRRK2 led to production of enlarged lysosomes and a reduction in lysosomal pH. The perturbations in lysosomal pH and morphology were rescued by selective inhibition of LRRK2's kinase activity. This suggests that the *G2019S LRRK2* mutation promotes lysosomal dysfunction (60).

The role of LRRK2 in autophagy has been controversial, as LRRK2 knockdown has been shown to both reduce and potentiate autophagic flux (61,62). Studies in mutant fibroblasts showed both

increased levels of autophagy through activation of the MEK/ERK pathway in G2019S-LRRK2 cells (63) and decreased responses to autophagy induction in R1441G, Y1699C and G2019S-LRRK2 cells. Studies of PD fibroblast-derived neurons (G2019S-LRRK2) also showed impairment of autophagy, however at the stage of autophagosome clearance level rather than induction. A recent study in an astrocyte cell model proposes that the kinase activity of LRRK2 negatively regulates autophagy, as chemical inhibition of LRRK2 kinase activity led to induction of non-canonical, mTOR/ULK1-independent, Beclin 1-dependent autophagy (64). LRRK2 studies have been complicated by the existence of a wide range of LRRK2 models and LRRK2 modulators and the role of LRRK2 in autophagy remains yet to be clarified.

Heterozygous mutations in the gene encoding the lysosomal enzyme glucocerebrosidase (GBA) are the most common known genetic risk factor for PD. Individuals carrying a heterozygous GBA mutation have a five-fold higher risk of developing PD than non-carriers (65). Homozygous mutations in this gene cause Gaucher disease (GD), in which the loss of GBA causes accumulation of its substrate glucosylceramide within lysosomes, leading to lysosomal dysfunction (66). Studies using iPSC-derived neurons from PD patients with GBA mutations showed increased α-synuclein levels as well as autophagic and lysosomal defects (67). In non-GBA related PD patients, α-syn accumulation impairs trafficking of GBA to lysosomes (68). This suggests a possible positive feedback loop, in which loss of GBA leads to impaired lysosomal function and α-syn accumulation. Inhibition of glycosylceramide synthase (the enzyme that regulates the formation of the GBA substrate glucocerebroside) in a mouse model of GBA-related synucleinophathy and in mice overexpressing α-syn (A53T) reduced α-syn and ubiquitin accumulation in hippocampal neurons and improved cognitive behaviour (69).

Mutations in the gene *ATP13A2/PARK9*, which encodes a transmembrane lysosomal P-type ATPase, cause familial Kufor-Rakeb syndrome characterized by early-onset Parkinsonism (70). Mutations in ATP13A2 impair lysosomal function, which manifests with an accumulation of lysosomes and autophagosomes (71). Studies in cell models of PD showed that the dopaminergic neuron loss caused by α-syn overexpression could be rescued by co-expression of ATP13A2. Additionally, knockdown of the ATP13A2 ortholog in *C. elegans* enhanced α-syn misfolding, suggesting a link between α-syn and ATP13A2 (72). This was further supported by a study in which loss of ATP13A2 *in vitro* impaired lysosomal function and led to α-syn accumulation (73). The increase in α-syn was, however, not observed in mice, in which loss of ATP13A2 caused endolysosomal abnormalities without disrupting α-syn levels (74). Depletion of ATP13A2 *in vitro* led to a decrease in the levels of another PD-associated gene, synaptotagmin 11 (*SYT11*). The

decrease in SYT11 can account for the lysosomal dysfunction and impaired autophagosome degradation resulting from ATP13A2 deficiency, since SYT11 overexpression in ATP13A2 knockdown cells was able to rescue the autophagy defects in these cells, suggesting that these proteins act in the same pathway (15).

Autophagy as a therapeutic strategy

There is compelling evidence suggesting that impaired trafficking to the lysosome is a common mechanism underlying PD pathogenesis and that the aggregate-prone α-syn is an autophagy substrate (21,75). Upregulation of autophagy for degradation of aggregate-prone proteins is a promising mechanism to protect cells against the toxicity of such proteins and is, therefore, a major therapeutic strategy that is being explored (76).

Autophagy can be upregulated by targeting either the mTOR-dependent or mTOR-independent pathway. The allosteric mTORC1 inhibitor rapamycin ameliorates toxicity in animal models of PD (77,78). Due to its non-ATP-competitive inhibitory properties, rapamycin possesses a safer profile than ATP-competitive mTOR inhibitors like Torin1. While rapamycin specifically inhibits mTORC1, Torin1 inhibits mTORC1 as well as mTORC2, which is a positive autophagy regulator, thus leading to neuronal toxicity rather than protection (77).

Several mTOR-independent compounds that stimulate autophagosome formation have been studied for their therapeutic potential (79). Some of those have been shown to induce autophagy by stimulating the AMPK pathway, including the FDA-approved compound trehalose, which induces autophagy and enhances clearance of α-syn both *in vitro* (80) and *in vivo* (81). Nilotinib, a tyrosine kinase inhibitor and AMPK activator protects against loss of DA neurons and improves motor behaviour in a mouse model of PD by accelerating autophagic clearance of α-syn (82).

At early stages of disease, pharmacological activation of autophagosome biogenesis may be neuroprotective. Such strategies have potential for treating PD variants where increased levels of α-syn caused by factors like SNCA multiplication is the underlying mechanism and impairs not only autophagosome formation (46), but also endosomal trafficking (83), chaperone-mediated autophagy (84) and mitochondrial fusion (85). Indeed, lentiviral delivery of Beclin 1, which is a part of the VPS34 complex, into α-syn transgenic mice reduced α-syn accumulation by inducing

autophagosome biogenesis and enhanced lysosomal activation (75). However, inducing autophagy may be detrimental, if the presence of α-syn containing inclusion bodies is due to impaired autophagosome clearance caused by compromised lysosomal function, as is the case for several PD variants. In PD cases with mutations causing impaired autophagosome clearance, an upstream induction of autophagosome biogenesis may cause a pathological accumulation of autophagosomes and exacerbate disease.

Since α-syn aggregation in some cases of PD is associated with reduced lysosomal capacity implied by the impaired trafficking of lysosomal hydrolases (86) and reduction of lysosomal markers in nigral DA neurons (87), enhancement of lysosomal capacity through strategies like gene therapy may be a promising strategy. A study conducted in a PD model with adeno-associated virus (AAV) vector-mediated overexpression of human wild-type (WT) α-syn in the rat midbrain showed that delivery of the TFEB gene prevents α-syn induced neurodegeneration (88).

Although autophagy upregulation is a strategy that holds great therapeutic potential, the side-effect profiles of many autophagy inducers make them unsuited for long-term treatment due to the caveats related to prolonged autophagy upregulation. Development of specialized autophagy modulators with tightly regulated mechanisms of action is desirable for effective and differential treatment of the subtypes of PD.

Concluding remarks

Treatment of PD is expected to become more personalized in the future, due to the heterogeneity in causal elements underlying the vast range of PD variants. This will be facilitated by understanding the genetic determinants and molecular mechanisms responsible for the clinical phenotype of each PD patient, in order to tailor the most appropriate treatment option towards the elements that specifically disrupt autophagy and α-syn clearance in each case.

Acknowledgements

We are grateful for funding to DCR from the UK Dementia Research Institute (funded by the MRC, Alzheimers Research UK and the Alzheimer's Society), Tau consortium, Alzheimer's Research UK, Wellcome Trust (Principal Research Fellowship to 095317/Z/11/Z), a Wellcome Trust Strategic Grant to Cambridge Institute for Medical Research (100140/Z/12/Z), Gates Cambridge Scholarship (CK), Korea-UK R&D Collaboration grant (HI14C2036 to MJL) of the Korea Health Industry Development Institute and the National Research Foundation grant (2016R1A2B2006507 to MJL).

Author contributions

All authors contributed to the manuscript. CK coordinated the first draft.

Conflict of interest

The authors declare no conflict of interest.

References

1. Cai Z, Zhao B, Li K, Zhang L, Li C, Quazi SH, et al. Mammalian target of rapamycin: A valid therapeutic target through the autophagy pathway for alzheimer's disease? Journal of Neuroscience Research. 2012. p. 1105–18.

2. Cuervo AM. Autophagy and aging. Trends Genet. 2009;24(12):604–12.

3. Stolz A, Ernst A, Dikic I. Cargo recognition and trafficking in selective autophagy. Nat Cell Biol. 2014;16(6):495–501.

4. Menzies FM, Fleming A, Caricasole A, Bento CF, Andrews SP, Jackson A, et al. Autophagy and Neurodegeneration : Pathogenic Mechanisms and Therapeutic Opportunities. Neuron. 2017;93:1015–34.

5. Hosokawa N, Hara T, Kaizuka T, Kishi C, Takamura A, Miura Y, et al. Nutrient-dependent mTORC1 Association with the ULK1–Atg13–FIP200 Complex Required for Autophagy. Mol Biol Cell. 2009;20:1981–91.

6. Ganley IG, Lam DH, Wang J, Ding X, Chen S, Jiang X. ULK1-ATG13-FIP200 Complex Mediates mTOR Signaling and Is Essential for Autophagy. J Biol Chem. 2009;284(18):12297–305.

7. Kim J, Kundu M, Viollet B, Guan K. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011;13(2):132–41.

8. Russell RC, Tian Y, Yuan H, Park HW, Chang Y, Kim H, et al. ULK1 induces autophagy by phosphorylating Beclin-1 and activating Vps34 lipid kinase. Nat Cell Biol. 2014;15(7):741–50.

9. Backer JM. The regulation and function of Class III PI3Ks: novel roles for Vps34. Biochem J. 2008;410:1–17.

10. Dooley HC, Razi M, Polson HEJ, Girardin SE, Wilson MI, Tooze SA. WIPI2 Links LC3 Conjugation with PI3P, Autophagosome Formation, and Pathogen Clearance by Recruiting Atg12–5-16L1. Mol Cell. 2014;55(2):238–52.

11. Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. Nat Cell Biol. 2010;12(8):747–57.

12. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol. 2008;182(4):685–701.

13. Hailey DW, Rambold AS, Satpute-krishnan P, Mitra K, Sougrat R, Kim PK. Mitochondria Supply Membranes for Autophagosome Biogenesis during Starvation. Cell. 2010;141(4):656–67.

14. Hanada T, Noda NN, Satomi Y, Ichimura Y, Fujioka Y, Takao T, et al. The Atg12-Atg5 Conjugate Has a Novel E3-like Activity for Protein Lipidation in Autophagy. J Biol Chem. 2007;282(52):37298–302.

15. Bento CF, Ashkenazi A, Jimenez-sanchez M, Rubinsztein DC. The Parkinson's disease-associated genes ATP13A2 and SYT11 regulate autophagy via a common pathway. Nat Commun. 2016;7(11803):1–16.

16. Martini-stoica H, Xu Y, Ballabio A. The Autophagy–Lysosomal Pathway in Neurodegeneration: A TFEB Perspective. Trends Neurosci. 2016;39(4):221–34.

17. Sardiello M, Palmieri M, Ronza A di, Medina DL, Valenza M, Gennarino VA, et al. A Gene Network Regulating Lysosomal Biogenesis and Function. Science (80-). 2009;325:473–6.

18. Tsunemi T, Ashe TD, Morrison BE, Soriano KR, Au J, Roque R a V, et al. PGC-1α rescues Huntington's disease proteotoxicity by preventing oxidative stress and promoting TFEB

function. Sci Transl Med. 2012;4(142):1–28.

19. Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, et al. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature. 2006;441(15):880–4.

20. Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-migishima R, et al. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nat Publ Gr. 2006;441(June):885–9.

21. Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. α-Synuclein Is Degraded by Both Autophagy and the Proteasome. J Biol Chem. 2003;278(27):25009–13.

22. Spillantini M, Schmidt M, Lee V, Trojanowski J, Jakes R, Goedert M. α-Synuclein in Lewy bodies. Nature. 1997;388:839–40.

23. Laar AD Van, Jain S. Non-motor Symptoms of Parkinson Disease: Update on the Diagnosis and Treatment. Neurologist. 2004;10(4):185–94.

24. Ross OA, Braithwaite AT, Skipper LM, Kachergus J, Hulihan MM, Middleton FA, et al. Genomic investigation of α-Synuclein multiplication and parkinsonism. J Natl Cancer Inst. 1998;90(24):1894–900.

25. Cuervo AM, Stefanis L, Fredenburg R, Lansbury PT, Sulzer D. Impaired Degradation of Mutant a-Synuclein by Chaperone-Mediated Autophagy. Science (80-). 2004;29831(1994):1292–5.

26. Tofaris GK, Tae H, Hourez R, Jung J, Pyo K, Goldberg AL. Ubiquitin ligase Nedd4 promotes α-synuclein degradation by the endosomal – lysosomal pathway. PNAS. 2011;108(41):17004–9.

27. Chang D, Nalls MA, Hallgrímsdóttir IB, Hunkapiller J, Brug M Van Der, Kerchner GA, et al. A meta-analysis of genome-wide association studies identifies 17 new Parkinson's disease risk loci. Nat Genet. 2017;49(10):1511–6.

28. Kitada T, Asakawa S, Hattori N, Matsumine H, Yamamura Y, Minoshima S, et al. Mutations in the parkin gene cause autosomal recessive juvenile parkinsonism. Nature. 1998;169(1993):166–9.

29. Clark IE, Dodson MW, Jiang C, Cao JH, Huh JR, Seol JH, et al. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature. 2006;441:1162–6.

30. Valente EM, Abou-sleiman PM, Caputo V, Muqit MMK, Harvey K, Gispert S, et al.

Hereditary Early-Onset Parkinson's Disease Caused by Mutations in PINK1. Science (80-). 2004;304:1158–61.

31. Kane LA, Lazarou M, Fogel AI, Li Y, Yamano K, Sarraf SA, et al. PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. J Cell Biol. 2014;205(2):143–53.

32. Pickrell AM, Youle RJ. The Roles of PINK1, Parkin, and Mitochondrial Fidelity in Parkinson's Disease. Neuron. 2015;85(2):257–73.

33. Lazarou M, Sliter DA, Kane LA, Sarraf SA, Burman JL, Sideris DP, et al. The ubiquitin kinase PINK1 recruits autophagy receptors to induce mitophagy. Nature. 2016;524(7565):309–14.

34. Von Coelln R, Thomas B, Savitt JM, Lim KL, Sasaki M, Hess EJ, et al. Loss of locus coeruleus neurons and reduced startle in parkin null mice. PNAS. 2004;101(29):10744–9.

35. Goldberg MS, Fleming SM, Palacino JJ, Cepeda C, Lam HA, Bhatnagar A, et al. Parkin-deficient Mice Exhibit Nigrostriatal Deficits but Not Loss of Dopaminergic Neurons. J Biol Chem. 2003;278(44):43628–35.

36. Itier J, Iba P, Cohen-salmon C, Bohme GA, Laville M, Pratt J, et al. Parkin gene inactivation alters behaviour and dopamine neurotransmission in the mouse. Hum Mol Genet. 2003;12(18):2277–91.

37. Kitada T, Pisani A, Karouani M, Haburcak M, Martella G, Tscherter A, et al. Impaired dopamine release and synaptic plasticity in the striatum of Parkin-/- mice. J Neurochem. 2009;110:613–21.

38. Gautier CA, Kitada T, Shen J. Loss of PINK1 causes mitochondrial functional defects and increased sensitivity to oxidative stress. PNAS. 2008;105(32):11364–9.

39. Stevens DA, Lee Y, Chul H, Dae B, Lee Y, Bower A. Parkin loss leads to PARIS-dependent declines in mitochondrial mass and respiration. PNAS. 2015;112(37):11696–702.

40. Pickrell AM, Huang C, Kennedy SR, Ordureau A, Sideris DP, Hoekstra JG, et al. Endogenous Parkin Preserves Dopaminergic Substantia Nigral Neurons following Mitochondrial DNA Mutagenic Stress. Neuron. 2016;87(2):371–81.

41. Shiba-Fukushima K, Ishikawa K, Inoshita T, Izawa N, Takanashi M, Sato S, et al. Evidence that phosphorylated ubiquitin signaling is involved in the etiology of Parkinson's disease. Hum Mol Genet. 2017;26(16):3172–85.

42. Burchell VS, Nelson DE, Sanchez-martinez A, Delgado- M, Ivatt RM, Pogson JH, et al. The Parkinson's disease genes Fbxo7 and Parkin interact to mediate mitophagy. Nat Neurosci. 2014;16(9):1–28.

43. Zhou ZD, Xie SP, Sathiyamoorthy S, Saw WT, Sing TY, Ng SH, et al. F-box protein 7 mutations promote protein aggregation in mitochondria and inhibit mitophagy. Hum Mol Genet. 2015;24(22):6314–30.

44. Tanik SA, Schultheiss CE, Volpicelli-daley LA, Brunden KR, Lee VMY. Lewy Body-like α-Synuclein Aggregates Resist Degradation and Impair Macroautophagy. J Biol Chem. 2013;288(21):15194–210.

45. Volpicelli-daley LA, Gamble KL, Schultheiss CE, Riddle DM, West AB, Lee VM, et al. Formation of α -synuclein Lewy neurite – like aggregates in axons impedes the transport of distinct endosomes. Mol Biol Cell. 2014;25:4010–23.

46. Winslow AR, Chen CW, Corrochano S, Acevedo-Arozena A, Gordon DE, Peden AA, et al. α-Synuclein impairs macroautophagy: Implications for Parkinson's disease. J Cell Biol. 2010;190(6):1023–37.

47. Gomez TS, Billadeau DD. A FAM21-Containing WASH Complex Regulates Retromer-Dependent Sorting. Dev Cell. 2009;17(5):699–711.

48. Seaman MNJ. The retromer complex - endosomal protein recycling and beyond. J Cell Sci. 2012;125(20):4693–702.

49. Zavodszky E, Seaman MN, Moreau K, Jimenez-Sanchez M, Breusegem SY, Harbour ME, et al. Mutation in VPS35 associated with Parkinson's disease impairs WASH complex association and inhibits autophagy. Nat Commun. 2014;5(3828):1–16.

50. Malik BR, Godena VK, Whitworth AJ. VPS35 pathogenic mutations confer no dominant toxicity but partial loss of function in Drosophila and genetically interact with parkin. Hum Mol Genet. 2015;24(21):6106–17.

51. Martinez A, Lectez B, Ramirez J, Popp O, Sutherland JD, Urbé S, et al. Quantitative proteomic analysis of Parkin substrates in Drosophila neurons. Mol Neurodegener. 2017;12(1):29.

52. Song P, Trajkovic K, Tsunemi T, Krainc D. Parkin Modulates Endosomal Organization and Function of the Endo-Lysosomal Pathway. J Neurosci. 2016;36(8):2425–37.

53. Follett J, Norwood SJ, Hamilton NA, Mohan M, Kovtun O, Tay S, et al. The Vps35 D620N

Mutation Linked to Parkinson's Disease Disrupts the Cargo Sorting Function of Retromer. Traffic. 2013;15(2):230–44.

54. Tang F-L, Erion JR, Tian Y, Liu W, Yin D-M, Ye J, et al. VPS35 in Dopamine Neurons Is Required for Endosome-to-Golgi Retrieval of Lamp2a, a Receptor of Chaperone-Mediated Autophagy That Is Critical for α-Synuclein Degradation and Prevention of Pathogenesis of Parkinson's Disease. J Neurosci. 2015;35(29):10613–28.

55. Dhungel N, Eleuteri S, Masliah E, Gitler AD, Dhungel N, Eleuteri S, et al. Parkinson's Disease Genes VPS35 and EIF4G1 Interact Genetically and Converge on a-Synuclein. Neuron. 2015;85(1):76–87.

56. Qiao L, Hamamichi S, Caldwell KA, Caldwell GA, Yacoubian TA, Wilson S, et al. Lysosomal enzyme cathepsin D protects against alpha-synuclein aggregation and toxicity. Mol Brain. 2008;1(17):1–18.

57. Dodson MW, Zhang T, Jiang C, Chen S, Guo M. Roles of the Drosophila LRRK2 homolog in Rab7-dependent lysosomal positioning. Hum Mol Genet. 2012;21(6):1350–63.

58. Dodson MW, Leung LK, Lone M, Lizzio MA, Guo M. Novel ethyl methanesulfonate (EMS) - induced null alleles of the Drosophila homolog of LRRK2 reveal a crucial role in endolysosomal functions and autophagy in vivo. Dis Model Mech. 2014;7:1351–63.

59. Macleod DA, Rhinn H, Kuwahara T, Zolin A, Paolo G Di, Mccabe BD, et al. RAB7L1 interacts with LRRK2 to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron. 2014;77(3):425–39.

60. Henry AG, Aghamohammadzadeh S, Samaroo H, Chen Y, Mou K, Needle E, et al. Pathogenic LRRK2 mutations, through increased kinase activity, produce enlarged lysosomes with reduced degradative capacity and increase ATP13A2 expression. Hum Mol Genet. 2015;24(21):6013–28.

61. Schapansky J, Nardozzi JD, Felizia F, Lavoie MJ. Membrane recruitment of endogenous LRRK2 precedes its potent regulation of autophagy. Hum Mol Genet. 2014;23(16):4201–14.

62. Alegre-Abarrategui J, Christian H, Lufino MMP, Mutihac R, Venda LL, Ansorge O, et al. LRRK2 regulates autophagic activity and localizes to specific membrane microdomains in a novel human genomic reporter cellular model. Hum Mol Genet. 2009;18(21):4022–34.

63. Pedro JMB-S, Niso-Santano M, Gómez-Sánchez R, Pizarro-Estrella E, Aiastui-Pujana A, Goristidi A, et al. The LRRK2 G2019S mutant exacerbates basal autophagy through

activation of the MEK/ERK pathway. Cell Mol Life Sci. 2013;70:121–36.

64. Manzoni C, Mamais A, Roosen DA, Dihanich S, Soutar MPM, Plun-favreau H, et al. mTOR independent regulation of macroautophagy by Leucine Rich Repeat Kinase 2 via Beclin-1. Sci Rep. 2016;6(35106):1–10.

65. Sidransky E, Lopez G. The link between the GBA gene and parkinsonism. Lancet Neurol. 2012;11:986–98.

66. Xu Y, Sun H, Quinn B, Witte D, Grabowski G. Accumulation and distribution of α-synuclein and ubiquitin in the CNS of Gaucher disease mouse models. Biophys Chem. 2005;257(5):2432–7.

67. Schöndorf DC, Aureli M, Mcallister FE, Hindley CJ, Mayer F, Scho DC, et al. iPSC-derived neurons from GBA1-associated Parkinson's disease patients show autophagic defects and impaired calcium homeostasis. Nat Commun. 2014;5(4028):1–17.

68. Mazzulli JR, Xu YH, Sun Y, Knight AL, McLean PJ, Caldwell GA, et al. Gaucher disease glucocerebrosidase and a-synuclein form a bidirectional pathogenic loop in synucleinopathies. Cell. 2011;146(1):37–52.

69. Sardi SP, Viel C, Clarke J, Treleaven CM, Richards AM, Park H. Glucosylceramide synthase inhibition alleviates aberrations in synucleinopathy models. PNAS. 2017;114(10):2699–704.

70. Ramirez A, Heimbach A, Gründemann J, Stiller B, Hampshire D, Cid LP, et al. Hereditary parkinsonism with dementia is caused by mutations in ATP13A2, encoding a lysosomal type 5 P-type ATPase. Nat Genet. 2006;38(10):1184–91.

71. Dehay B, Ramirez A, Martinez-vicente M, Perier C, Canron M. Loss of P-type ATPase ATP13A2/PARK9 function induces general lysosomal deficiency and leads to Parkinson disease neurodegeneration. Proc Natl Acad Sci U S A. 2012;109(24):9611–6.

72. Gitler AD, Chesi A, Geddie ML, Strathearn KE, Hamamichi S, Hill KJ, et al. Alpha-synuclein is part of a diverse and highly conserved interaction network that includes PARK9 and manganese toxicity. Nat Genet. 2009;41(3):308–15.

73. Usenovic M, Tresse E, Mazzulli JR, Taylor JP, Krainc D. Deficiency of ATP13A2 Leads to Lysosomal Dysfunction, α-Synuclein Accumulation, and Neurotoxicity. J Neurosci. 2012;32(12):4240–6.

74. Kett LR, Stiller XB, Bernath MM, Tasset I, Blesa X, Jackson-lewis V, et al. α-Synuclein-

Independent Histopathological and Motor Deficits in Mice Lacking the Endolysosomal Parkinsonism Protein Atp13a2. 2015;35(14):5724–42.

75. Spencer B, Potkar R, Trejo M, Rockenstein E, Gindi R, Adame A, et al. Beclin 1 Gene Transfer Activates Autophagy and Ameliorates the Neurodegenerative Pathology in α-Synuclein Model of Parkinson's and Lewy Body Disease. J Neurosci. 2010;29(43):13578–88.

76. Harris H, Rubinsztein DC. Control of autophagy as a therapy for neurodegenerative disease. Nat Rev. 2011;8(2):108–17.

77. Malagelada C, Jin ZH, Jackson-lewis V, Przedborski S, Greene LA. Rapamycin Protects against Neuron Death in In Vitro and In Vivo Models of Parkinson's Disease. J Neurosci. 2010;30(3):1166–75.

78. Crews L, Spencer B, Desplats P, Patrick C, Paulino A, Rockenstein E, et al. Selective Molecular Alterations in the Autophagy Pathway in Patients with Lewy Body Disease and in Models of a -Synucleinopathy. PLoS One. 2010;5(2).

79. Renna M, Jimenez-sanchez M, Sarkar S, Rubinsztein DC. Chemical Inducers of Autophagy That Enhance the Clearance of Mutant Proteins in Neurodegenerative Diseases. J Biol Chem. 2010;285(15):11061–7.

80. Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinsztein DC. Trehalose, a Novel mTOR-independent Autophagy Enhancer, Accelerates the Clearance of Mutant Huntingtin. J Biol Chem. 2007;282(8):5641–52.

81. Tanji K, Miki Y, Maruyama A, Mimura J, Matsumiya T, Mori F, et al. Biochemical and Biophysical Research Communications Trehalose intake induces chaperone molecules along with autophagy in a mouse model of Lewy body disease. Biochem Biophys Res Commun. 2015;465(4):746–52.

82. Hebron ML, Lonskaya I, Moussa CE. Nilotinib reverses loss of dopamine neurons and improves motor behavior via autophagic degradation of α-synuclein in Parkinson's disease models. Hum Mol Genet. 2013;22(16):3315–28.

83. Soper JH, Kehm V, Burd CG, Bankaitis VA, Lee VM-Y. Aggregation of α-Synuclein in S. cerevisiae is Associated with Defects in Endosomal Trafficking and Phospholipid Biosynthesis. J Mol Neurosci. 2011;43(3):391–405.

84. Xilouri M, Vogiatzi T, Vekrellis K, Park D, Stefanis L. Abberant α-Synuclein Confers Toxicity

to Neurons in Part through Inhibition of Chaperone-Mediated Autophagy. PLoS One. 2009;4(5):16–20.

85. Kamp F, Exner N, Lutz AK, Wender N, Hegermann J, Brunner B, et al. Inhibition of mitochondrial fusion by α-synuclein is rescued by PINK1, Parkin and DJ-1. EMBO J. 2010;29(20):3571–89.

86. Mazzulli JR, Zunke F, Isacson O, Studer L, Krainc D. α-Synuclein–induced lysosomal dysfunction occurs through disruptions in protein trafficking in human midbrain synucleinopathy models. PNAS. 2016;113(7):1931–6.

87. Chu Y, Dodiya H, Aebischer P, Olanow CW, Kordower JH. Alterations in lysosomal and proteasomal markers in Parkinson's disease: Relationship to alpha-synuclein inclusions. Neurobiol Dis. 2009;35(3):385–98.

88. Decressac M, Mattsson B, Weikop P, Lundblad M, Jakobsson J, Björklund A. TFEB-mediated autophagy rescues midbrain dopamine neurons from α -synuclein toxicity. PNAS. 2013;110(19):1817–26.

Figure Legends:

Figure 1. Overview of the autophagy pathway. Low levels of nutrients, growth factors and energy levels culminate in inhibition of mTORC1 and AMPK activation. AMPK activates the ULK1 complex, which subsequently activates the VPS34 complex, resulting in PI3P synthesis. PI3P recruits WIPI2 and assists in the recruitment of the ATG12-ATG5-ATG16L1 complex, which is essential for the conjugation of LC3-I to PE, leading to membrane elongation and engulfment of cytosolic contents. These include aggregate-prone proteins and damaged organelles that ultimately are degraded in the lysosome after fusion with autophagosomes. Upregulation of autophagy after starvation/mTOR inhibition is in part regulated by translocation of TFEB to the nucleus, where it induces transcription of many autophagic and lysosomal genes. AMPK, AMP-dependent protein kinase; FIP200, focal adhesion kinase family interacting protein of 200kD; mTORC1, mammalian target of rapamycin complex 1; PE, phosphatidylethanolamine; PI3P, phosphatidylinositol 3-phosphate; TFEB, transcription factor EB; ULK, mammalian homologs of the C. elegans uncoordinated-51 kinase.

Figure 2. PD-related genes associated with the autophagic and endolysosomal pathways. A variety of genes associated with sporadic and familial forms of PD are known to affect mitophagy, autophagosome biogenesis, lysosomal function and lysosome formation.

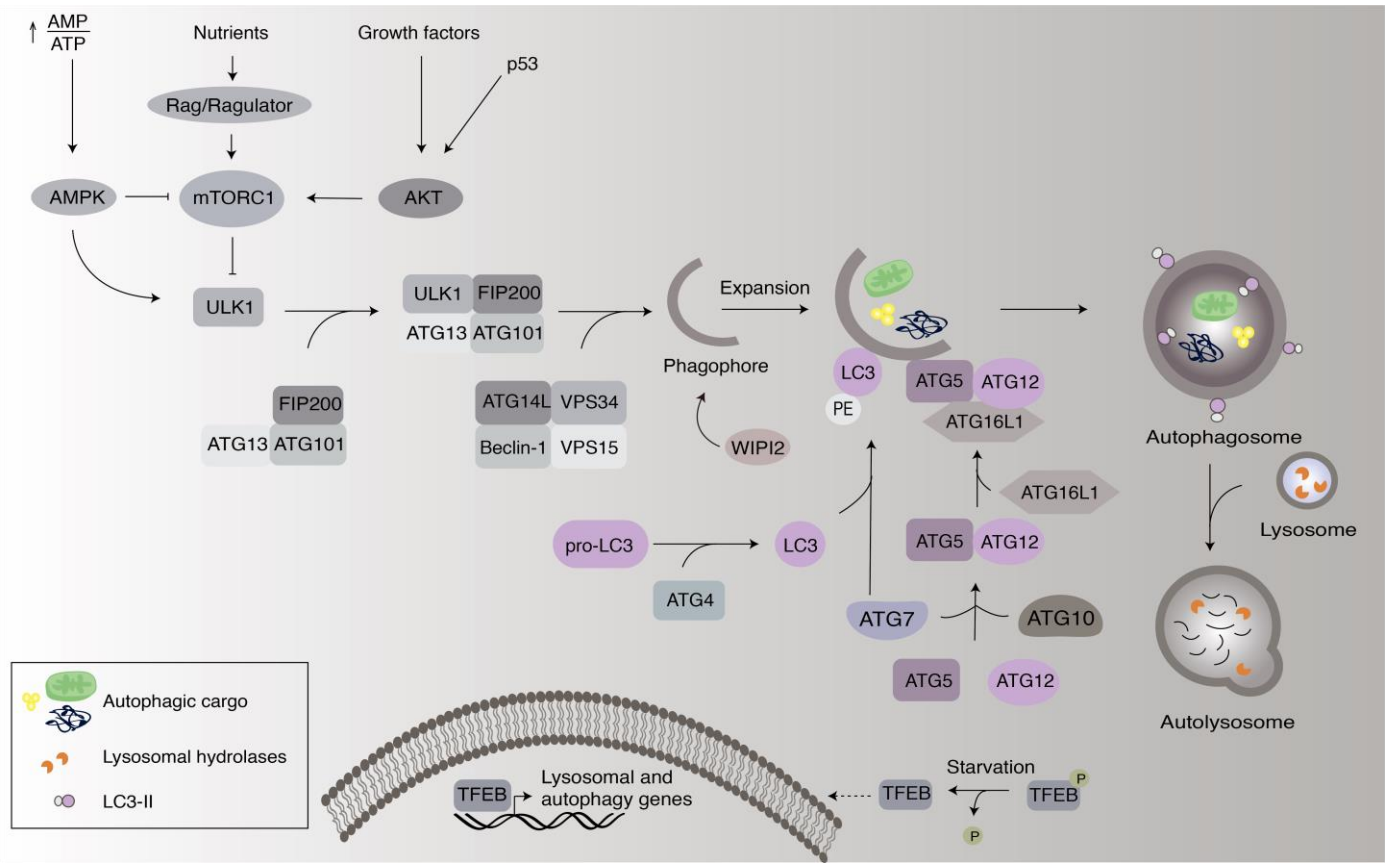
AMP
ATP
Nutrients
Growth factors
p53
Rag/Ragulator
AMPK
mTORC1
AKT
ULK1
ULK1
FIP200
ATG13
ATG101
FIP200
ATG13
ATG101
ATG14L
VPS34
Beclin-1
VPS15
WIPI2
Phagophore
Expansion
LC3
PE
ATG5
ATG12
ATG16L1
Autophagosome
Lysosome
ATG16L1
pro-LC3
LC3
ATG4
ATG5
ATG12
ATG7
ATG10
ATG5
ATG12
Autolysosome
Autophagic cargo
Lysosomal hydrolases
LC3-II
TFEB
Lysosomal and
autophagy genes
TFEB
Starvation
TFEB
P
P

Figure 1

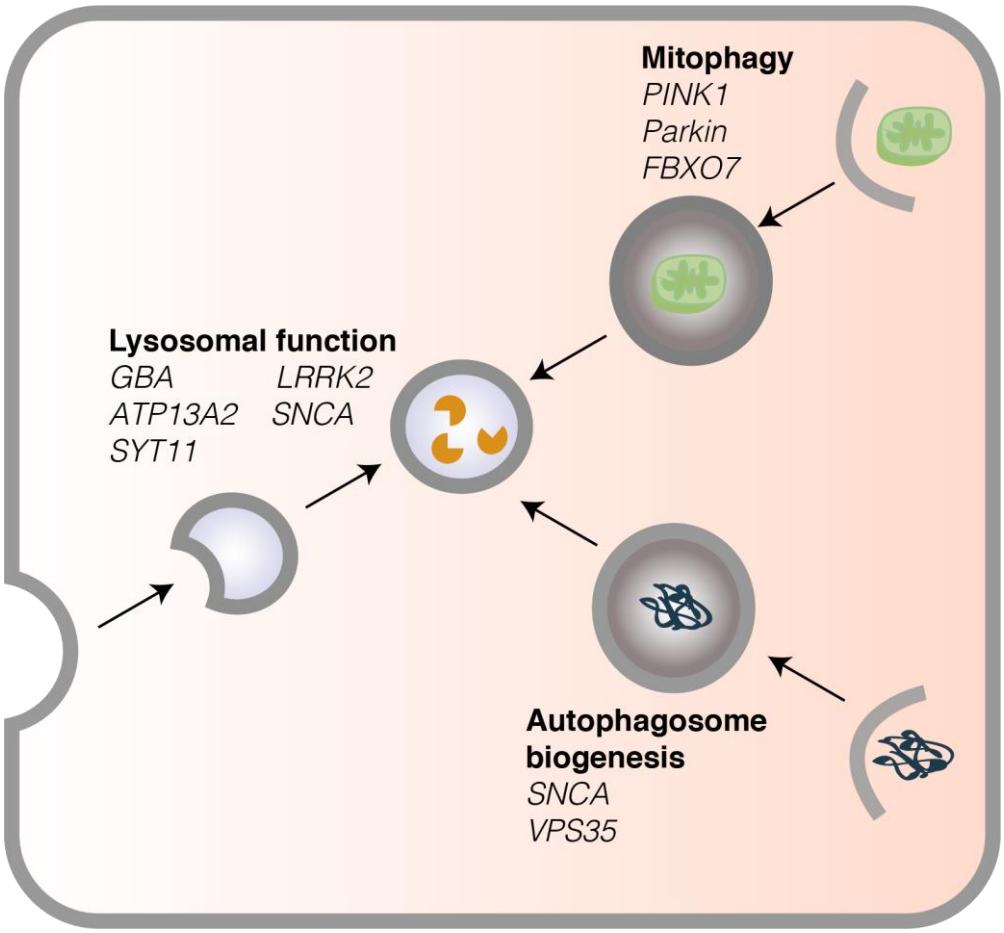
Mitophagy
PINK1
Parkin
FBXO7
Lysosomal function
GBA
ATP13A2
SYT11
LRRK2
SNCA
Autophagosome
biogenesis
SNCA
VPS35

Figure 2